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The Histogenesis of Hamster Pancreatic Cancer Induced by N-nitroso-bis(2-hydroxypropyl)amine

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Summary

Syrian golden hamsters were treated with weekly subcutaneous injection of 125 mg/kg body weight (Group 1), 250 mg/kg body weight (Group 2) or 500 mg/kg body weight (Group 3) of N-nitroso-bis(2-hydroxypropyl)amine (DHPN) and were sacrificed at 5, 10, 15, 25, 35 weeks or when moribound.

Five weeks later, focal hypertrophy appeared at a small area of a pancreatic duct and only one intralobular ductule. In Group 3, the intralobular ductule showed multilayer hyperplasia. The interlobular ductule and pancreatic duct showed unilayer hyperplasia.

Ten weeks later, small multiplication appeared. A restricted area of a pancreatic duct showed intraductal carcinoma. The common duct showed focal hypertrophy. An intrainsular glandular structure was seen.

Fifteen weeks later, hypertrophic epithelial multiplication appeared. Perinsular ductule proliferation was seen. The hyperplastic or multiplicative lesions extended to whole pancreas.

Twenty-seven weeks later, a few interlobular ductules showed intraductal carcinoma. Flat epithelial multiplication became adenoma consisting of about 20 locuses. Many hypertrophic and malignant altered epithelial multiplications appeared and surrounded by destructed parenchymal tissue, lymphocytic reaction and stromal proliferation. They became carcinoma *in situ*. In succession, carcinoma *in situ* became small adenocarcinoma. Finally, adenocarcinoma consisting of glands and mature desmoplastic reaction appeared.

Introduction

Since the best way against pancreatic cancer is recently to diagnose it as early as possible and to resect it completely. To know the initial site in its carcinogenesis and the mode of the spread-

Key words: Pancreatic cancer, Histogenesis, Experimental animal model, N-nitroso-bis(2-hydroxypropyl)amine, Syrian golden hamster.
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ing is very important[8]. Because of few cases of human early pancreatic cancer, the histogenesis of hamster pancreatic cancer model induced by N-nitroso-bis(2-hydroxypropyl)amine[2,6,7] or N-nitroso-bis(2-oxopropyl)amine (BOP)[10,11,13,14,18,19] is analized instead of them. In spite of incompletion of them as a model of human pancreatic cancer, analysis of the histogenesis of hamster pancreatic cancer model must give a clue to understanding the histogenesis of human pancreatic cancer[1,3,4].

Materials and Methods

Four groups of randomly bred, 8-week-old Syrian golden hamsters from the Nihon Dobutsu were used. They were kept under standard condition (room temperature, 24±2°C, humidity, 60±10%) in cages in groups of 5 by sex and given pellet diet for breeding F-2 (Funahashi farm) and water *ad libitum*. Each groups of 5 females and 5 males received weekly subcutaneous injection of N-nitroso-bis(2-hydroxypropyl)amine in 1 ml of olive oil, 125 mg/kg body weight (Group 1), 250 mg/kg body weight (Group 2) and 500 mg/kg body weight (Group 3). Controls (Group 4) were given solvent only. Animals were sacrificed at 5, 10, 15, 25, 35 weeks after the first injection or when moribound by i.p. injection of 0.5 ml Somnopentyl. Routine autopsies were performed at all dead animals and macroscopical findings were observed. The pancreases were fixed in 10% folmalin and processed for histology by conventional methods. The pancreases cut into 10 step sections were stained with hematoxylin and eosin. The durations stated for the latencies are from the beginning of treatment. Findings noted among groups and sexes were mentioned.

Results

Five Weeks Later. In a male of Group 1, a circumscribed area of the gastric duct and secondary duct at body showed unilayer hyperplasia (Fig. 1). This lesion was also seen at the splenic duct of a female of Group 1.

In a male of Group 2, a few intrainsular acinar cells and enlarged islets were seen. The splenic duct at body showed unilayer hyperplasia.

In a female of Group 2, a few islets showed budding and pumpkin shape composed of hyperplasia (Fig. 2). A restricted area of the lining epithelium of only one intralobular ductule at body of the gastric lobe showed metaplasia (Fig. 3).

In a male of Group 3, the connection of surrounding ductules to the islets lied scattered in the splenic lobe. A few intralobular ductules and interlobular ductules at body showed unilayer hyperplasia. Ergastroplasm-not-well-stained lobules appeared at tail. In the gastric lobe, the pancreatic duct and secondary duct at body showed unilayer hyperplasia and were surrounded by lymphocytic reaction (Fig. 4). At tail, most of intralobular ductules showed multilayer hyperplasia surrounded by lymphocytic reaction. The interlobular ductules showed multilayer hyperplasia (Fig. 5). Ergastroplasm-not-well-stained lobules appeared. In some of the lobules, parenchymal tissue was destructed and surrounded by lymphocytic reaction. A few intralobular

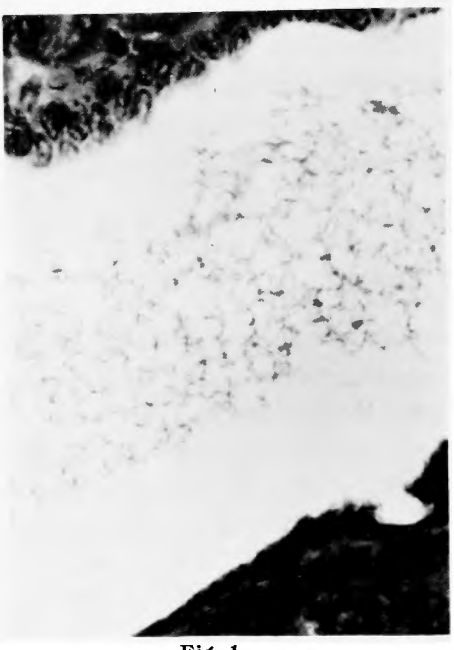
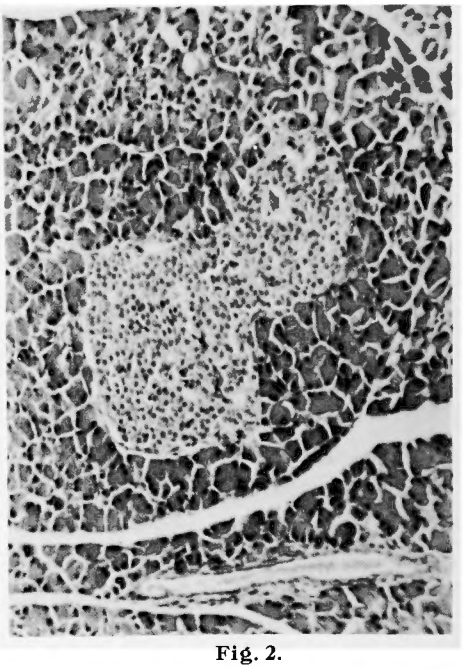


Fig. 1.



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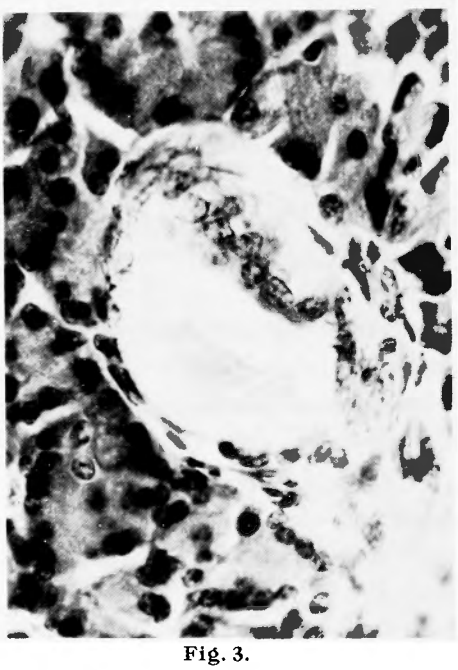

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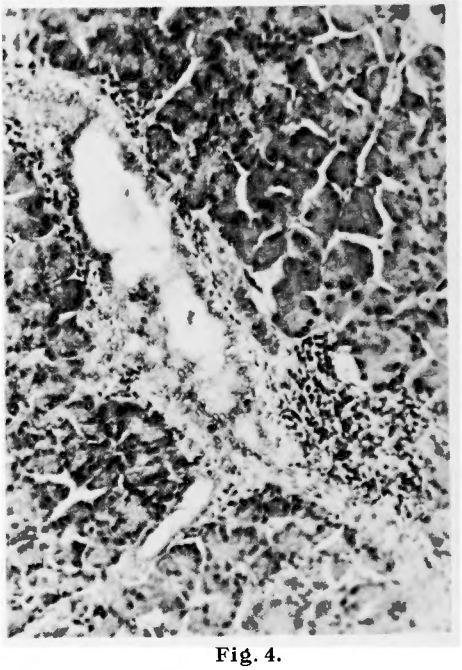

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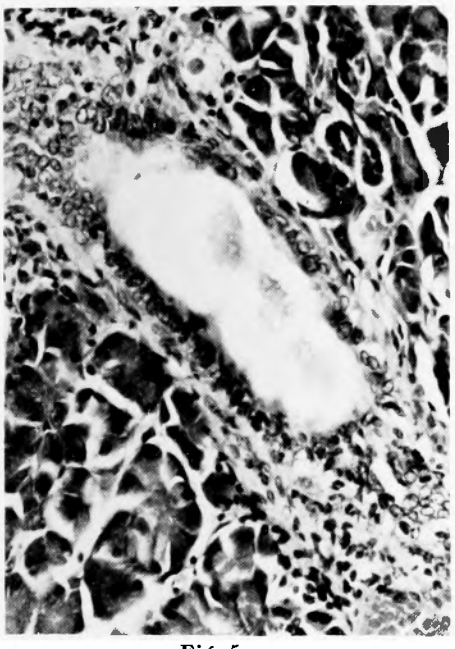

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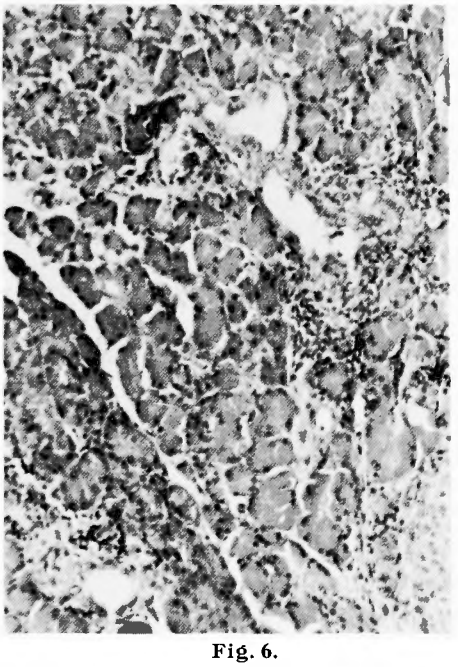

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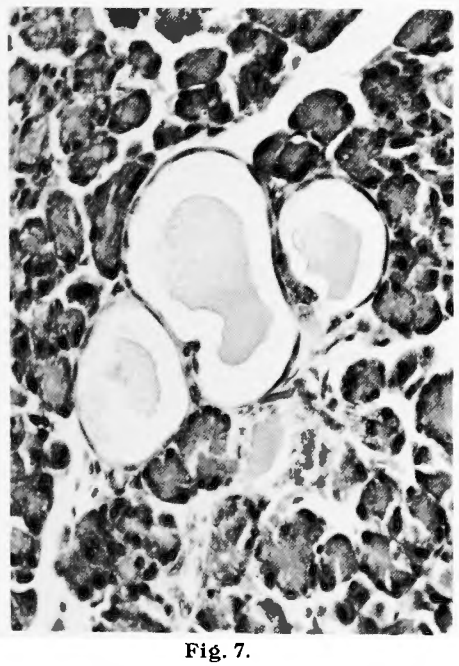

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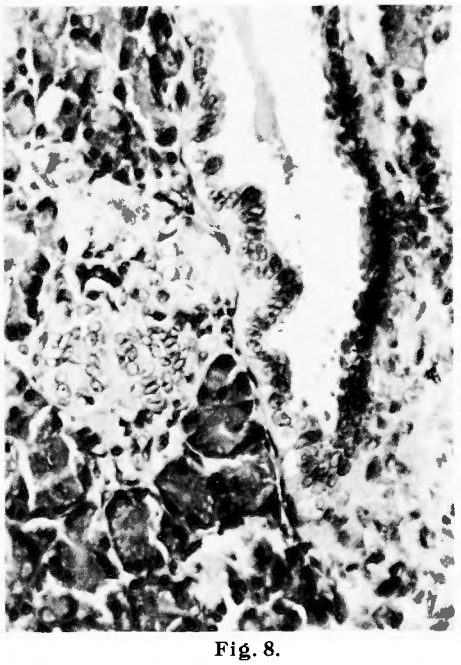

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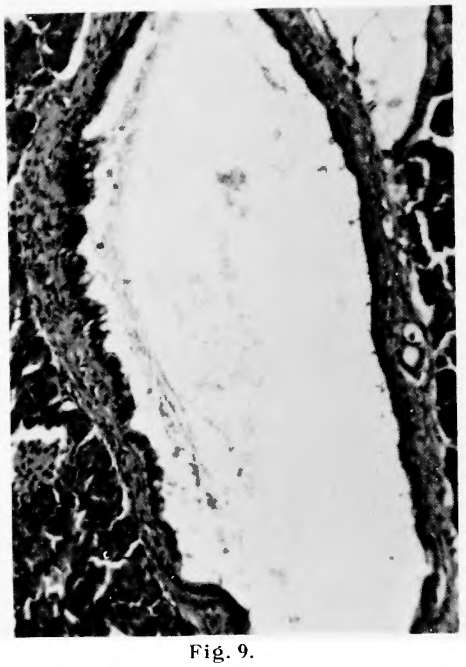
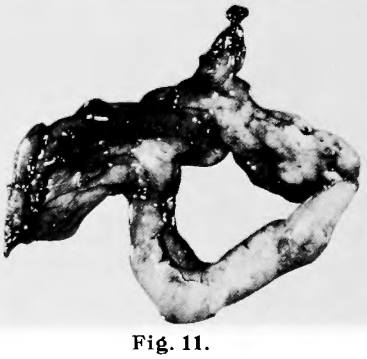


Fig. 9.

Fig. 10.



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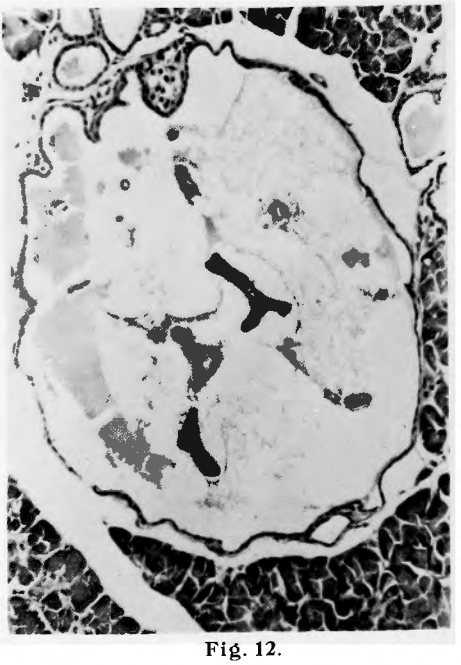

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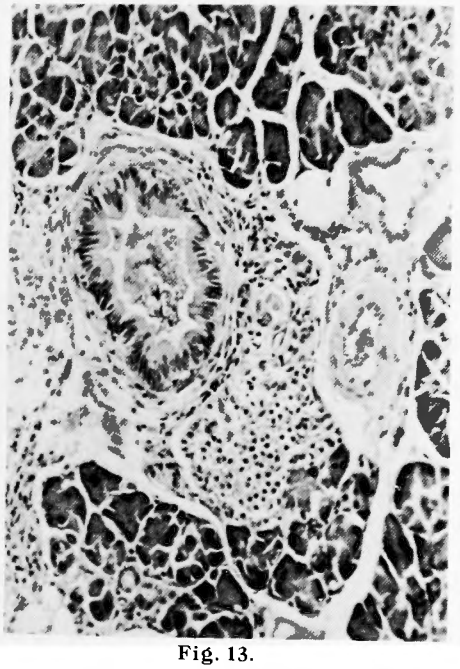

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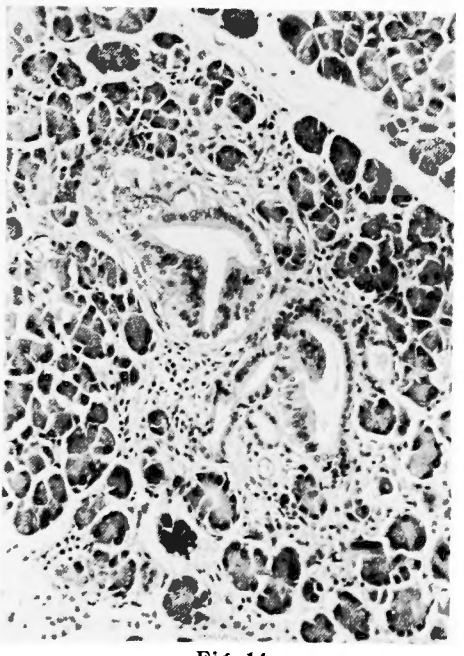

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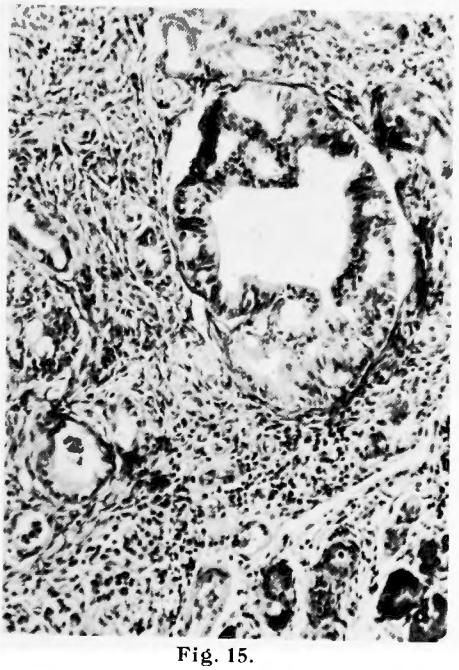

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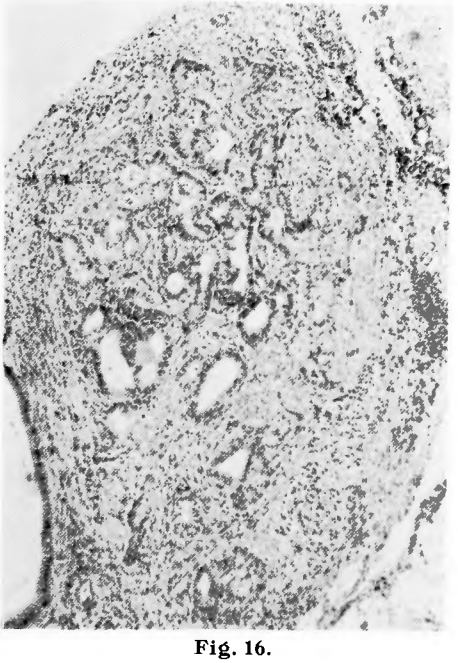

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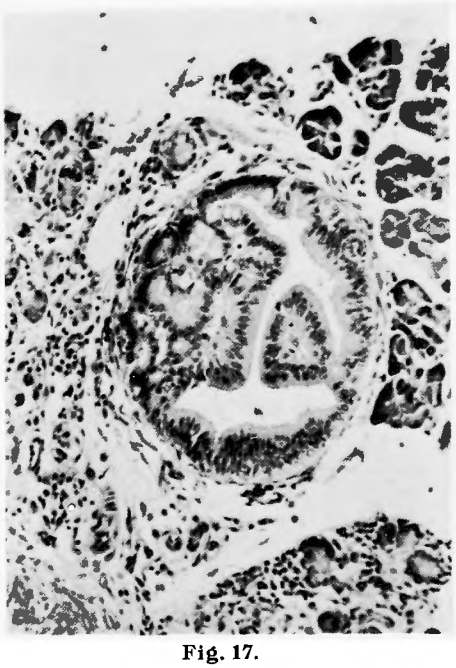

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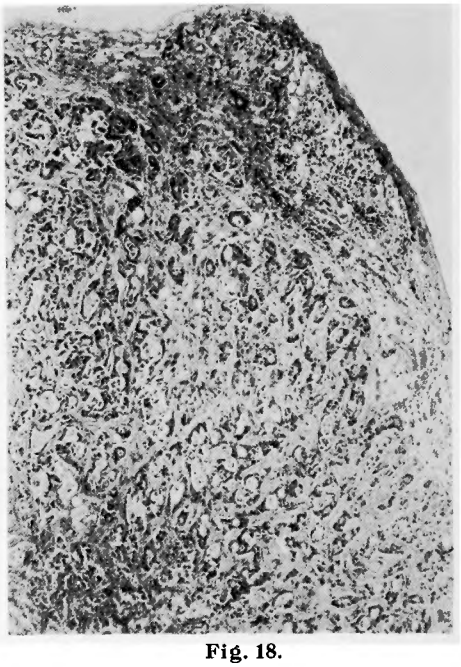

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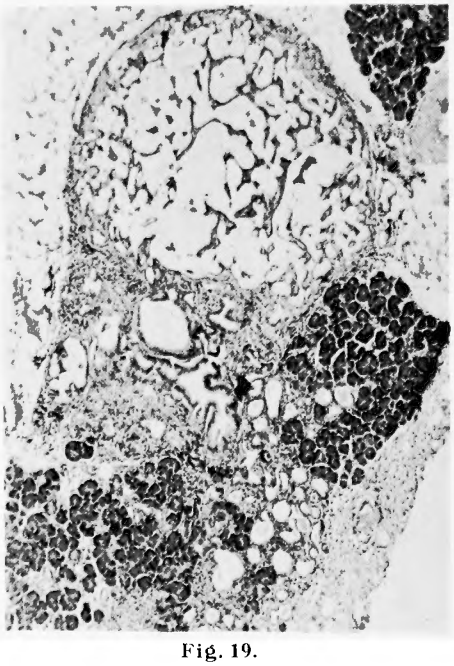

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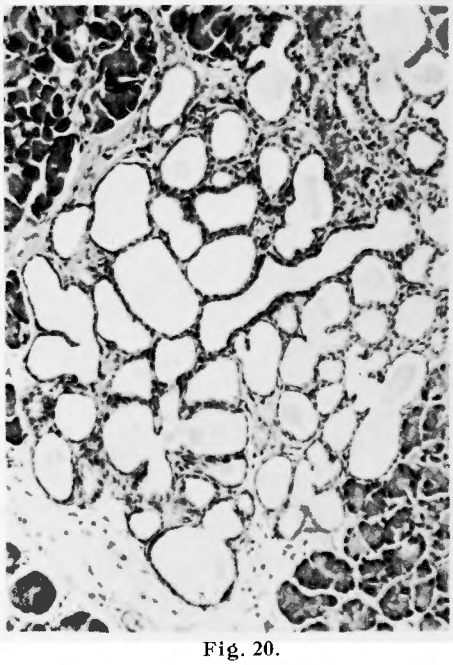

Fig. 20.

Fig. 21.

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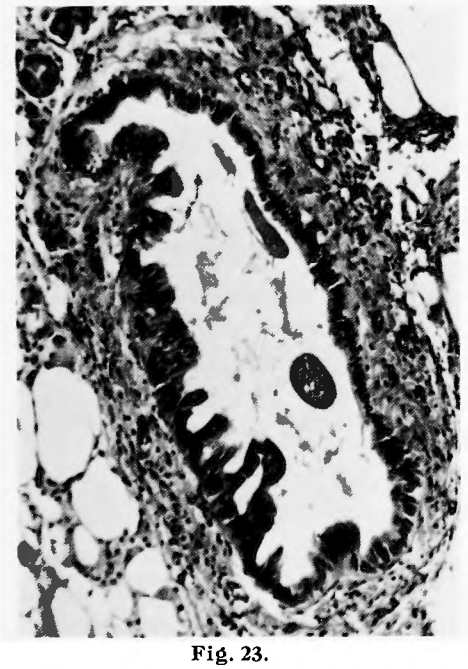

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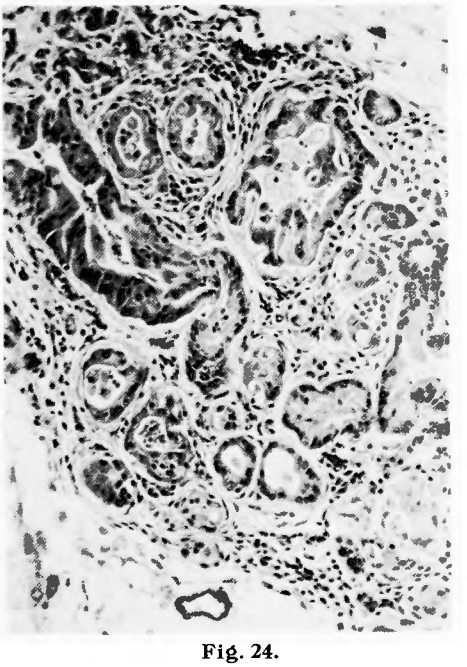

Fig. 24.

Fig. 1. Focal hypertrophy of a pancreatic duct. Cubic cells stand in a line regularly at lower epithelium but enlarged and atypical cells stand in a line irregularly at upper epithelium. Male, 5 weeks, Group 1, ×200.

Fig. 2. An enlarged islet seems to be composed of fusion of two islets. Female, 5 weeks, Group 2, ×50.

Fig. 3. Metaplasia of epithelium (upper) of an intralobular ductule. Female, 5 weeks, Group 2, ×200.

Fig. 4. Entire epithelium of a pancreatic duct (middle) and a secondary duct (lower left) shows unilayer hyperplasia surrounded by lymphocytic reaction (middle right). Male, 5 weeks, Group 3, ×100.

Fig. 5. The interlobular ductule shows multilayer hyperplasia. A vessel is seen at under in the interlobular septum. Male, 5 weeks, Group 3, ×100.

Fig. 6. The intralobular ductules at upper right show multilayer hyperplasia with cystic distension surrounded by lymphocytic reaction. In the upper lobe, ergastroplasm of the acinar cells is not well stained. Male, 5 weeks, Group 3, ×50.

Fig. 7. A flat epithelial multiplication consist of 3 locuses. An intralobular ductule is adjacent to it. Female, 10 weeks, Group 2, ×100.

Fig. 8. Intraductal carcinoma of a pancreatic duct does not proliferate into lumen exceedingly but spreads to surrounding connective tissue beneath the basement membrane. Female, 10 weeks, Group 2, ×100.

Fig. 9. The common duct shows focal hypertrophy at left. Female, 10 weeks, Group 2, ×50.

Fig. 10. An intrainsular glandular structure. The islet cells are seen at lower right. Male, 10 weeks, Group 3, ×50.

Fig. 11. The short segment, the duodenal lobe, is located laterally to the descending duodenum seemed intact. The splenic lobe at upper is white, uneven, firm and bearing small tumor nodules. The gastric lobe at lower is also white, uneven and firm. Female, 27 weeks, Group 1.

Fig. 12. Proliferation and cystic distension of the intrainsular and extrainsular ductules. Gradual atrophy of involved islets may be visualized in this figure. The final stage of islet cell atrophy shows the formation of trabeculae extending into cystic space. Eosinophilic substance in it indicates its communication to the surrounding intralobular ductule. Female, 27 weeks, Group 1, ×50.

Fig. 13. Marked hyperplasia of a periinsular ductule. Female, 10 weeks, Group 2, ×50.

Fig. 14. Ductular proliferation with atypical pattern. Cellular pleomorphism, loss of cell polarity are present. Such lesions seems to extend with affecting adjacent tissue. A few mitoses can be seen at this stage of hyperplasia. Female, 27 weeks, Group 1, ×50.

Fig. 15. Isolated lesion composed of irregular structures, destructed parenchymal tissue and lymphocytic reaction representing carcinoma *in situ*. Female, 27 weeks, Group 1, ×50.

Fig. 16. Small adenocarcinoma (in tail of the splenic lobe) located near a main duct (lower left) as in majority of adenocarcinomas. Female, 27 weeks, Group 1, ×20.

Fig. 17. More common type of intraductal carcinoma showing predominantly glans-within-a-gland structure. Such a pattern can be occasionally retained in adenocarcinomas deriving from these lesions. Female, 27 weeks, Group 1, ×50.

Fig. 18. Well differentiated adenocarcinoma showing a variety of glands and desmoplastic reaction encapsulated by connective tissue capsule with abundant capillaries. Female, 29 weeks, Group 1, ×20.

Fig. 19. A large cyst with trabecular structure (upper) and proliferation of islet cells, intrainsular and extrainsular ductules (middle) are seen. Female, 29 weeks, Group 2, ×20.

Fig. 20. Adenoma composed of ductules of various calibers. Epithelial lining is flat and uniform. The size of this adenoma exceeds that of the pancreatic lobule. Female, 29 weeks, Group 2, ×50.

Fig. 21. Pseudoductular transformation of exocrine acini is seen. In the acinus at upper left, acinar cells remain its natural pattern of basophilic and polygonal shape at upper but form ductular pattern at lower. Female, 29 weeks, Group 2, ×200.

Fig. 22. This pancreatic duct shows focally multilayer hyperplasia with goblet cell metaplasia. Female, 29 weeks, Group 2, ×100.

Fig. 23. The common duct shows multilayer hyperplasia. Female, 29 weeks, Group 2, ×50.

Fig. 24. In this carcinoma *in situ*, the epithelium of glands shows squamous-cell-like proliferation. Female, 35 weeks, Group 3, ×50.

ductules in the lobules distended and formed cysts (Fig. 6).

In a female of Group 3, enlarged islets lied scattered.

Ten Weeks Later. In a female of Group 1, enlarged islets slightly increased. The gastric duct at head showed unilayer hyperplasia.

In a female of Group 2, enlarged islets slightly increased. Neisidoblastosis from an intralobular ductule was seen. Flat epithelial multiplication consisting of a few locuses appeared (Fig. 7). The splenic duct at body showed intraductal carcinoma infiltrating to the surrounding connective tissue with destroying the basement membrane (Fig. 8). The common duct showed focal hypertrophy (Fig. 9).

In a male of Group 3, an islet at body of the splenic lobe showed an intrainsular glandular structure (Fig. 10). Most of the intralobular ductules showed unilayer hyperplasia. Ergastroplasm-not-well-stained lobules appeared. At head and body of the gastric lobe, the lobules with destructed parenchymal tissue and lymphocytic reaction appeared. In the lobules, the interlobular ductules showed multilayer hyperplasia and the acinar cells showed pseudoductular transformation. Most of the intralobular ductules and interlobular ductules showed unilayer hyperplasia or multilayer hyperplasia. The gastric duct at head showed multilayer hyperplasia.

In a female of Group 3, the pancreatic duct showed unilayer hyperplasia.

Fifteen Weeks Later. In a female of Group 1, enlarged islets lied scattered.

In a male of Group 3, in the splenic lobe, almost all parenchymal tissue was destructed and infiltrated with lymphocytic reaction. All intralobular ductules and interlobular ductules showed multilayer hyperplasia or atypical hyperplasia. A small number of hypertrophic epithelial multiplications appeared. In the gastric lobe, the pancreatic duct at body showed multilayer hyperplasia with marked atypia. The surrounding parenchymal tissue was destructed and infiltrated with numerous lymphocytic reaction. Adipose atrophy of parenchymal tissue was also seen.

In a female of Group 3, enlarged islets lied scattered. The islets at body of the gastric lobe showed intrainsular cystic distension. A few periinsular ductule proliferations were also seen. A few intralobular ductules and interlobular ductules showed unilayer hyperplasia. A small number of flat epithelial multiplications consisting of about 10 locuses and a few hypertrophic epithelial multiplications lied scattered in whole pancreas. The common duct showed atypical hyperplasia.

Sixteen Weeks Later. In a female of Group 3, a few intralobular ductules showed unilayer hyperplasia and a small number of them showed flat epithelial multiplication. A few interlobular ductules showed unilayer hyperplasia. The gastric duct showed intraductal carcinoma.

Twenty-two Weeks Later. In a male of Group 2, a few islets showed intrainsular glandular structures, intrainsular cystic distension and periinsular ductule proliferation. Eight per cent of the intralobular ductules showed unilayer hyperplasia. Eighteen per cent of them showed flat epithelial multiplication. Hypertrophic epithelial multiplications increased to 34%. A few interlobular ductules showed unilayer hyperplasia.

Twenty-three Weeks Later. In a male of Group 2, 15% of the intralobular ductules

showed unilayer hyperplasia and 27% of them showed flat epithelial multiplication. Seven per cent of them showed hypertrophic epithelial multiplication. A few interlobular ductules showed unilayer hyperplasia. A restricted area of the splenic duct at body showed multilayer hyperplasia. Mitosis of the acinar cells lied scattered.

Twenty-seven Weeks Later. In a female of Group 1, grossly the splenic lobe was white, uneven, firm and bearing small tumor nodules and the gastric lobe was also white, uneven and firm (Fig. 11). Histopathologically, 3 islets at body and tail of the gastric lobe showed intrainsular cystic distension. The final stage of islet cell atrophy was the formation of trabeculae extending into cystic spaces (Fig. 12). Three islets showed periinsular ductule proliferation. The lining epithelium of one of them showed atypical hyperplasia (Fig. 13). Thirty-seven per cent of the intralobular ductules showed unilayer hyperplasia. Three per cent of them showed multilayer hyperplasia. Eighteen per cent of them showed flat epithelial multiplication. Adenomas consist of about 20 locuses appeared. The epithelium of some multiplication became hypertrophic, increased atypia and surrounded by stromal proliferation (Fig. 14). Carcinoma *in situ* with malignant epithelial glands, lymphocytic reaction and stromal proliferation appeared at tail of the gastric lobe (Fig. 15). Small adenocarcinoma with desmoplastic reaction (Fig. 16) appeared at tail of the splenic lobe surrounded by atypical hyperplasia of the splenic duct and intraductal carcinoma of an interlobular ductule (Fig. 17). Twenty-seven per cent of the interlobular ductules showed multilayer hyperplasia. A few of the interlobular ductules showed intraductal carcinoma. The common duct showed atypical hyperplasia.

Twenty-nine Weeks Later. In a female of Group 1, a macroscopical tumor nodule was seen. Histopathologically, it was well differentiated adenocarcinoma (Fig. 18).

In a female of Group 2, the pattern of cancerous lesions resembled 27 weeks later of a female of Group 1. At head of the gastric lobe, a region composed of a large cyst, islet cells hyperplasia and proliferated glands appeared (Fig. 19). Three per cent of the intralobular ductules showed unilayer hyperplasia. Twenty-two per cent of them showed flat epithelial multiplication. A small number of adenomas consisting of 20 to 50 locuses lied scattered in whole pancreas (Fig. 20). A small number of pseudoductular transformation of exocrine acini were observed (Fig. 21). This figure was seen only in this hamster. Twenty-three per cent of the intralobular ductules showed hypertrophic epithelial multiplication. Carcinoma *in situ* was seen at head of the duodenal lobe and splenic lobe. Fifteen per cent of the interlobular ductules showed unilayer hyperplasia and 9% of them showed multilayer hyperplasia. The gastric duct at body showed multilayer hyperplasia with goblet cell metaplasia (Fig. 22). The common duct showed multilayer hyperplasia (Fig. 23).

Thirty-five Weeks Later. In a female of Group 3, 53% of the intralobular ductules showed hypertrophic epithelial multiplication. Adenomas were also seen. In carcinoma *in situ* at head of the splenic lobe, the epithelium of the glands showed squamous cell-like proliferation (Fig. 24).

Discussion

At 1974, POUR et al synthesized N-nitroso-bis(2-hydroxypropyl)amine from diisopropilnitrosamine derivative and induced a good experimental hamster pancreatic cancer model by it[14]. At 1977, they synthesized superior, N-nitroso-bis(2-oxopropyl)amine[12,15]. The histogenesis of hamster pancreatic cancer induced by them were analized.

ALTHOFF J.[2] mentioned as follows. The neoplasms originated from the ductal epithelium and developped progressively, adenomas were lined by epithelium of differing cells types, ranging from a flat singly ciliated form to cuboidal-columnar types, or to mixed cell populations. The epithelial lining of the ductal carcinomas exihibits tubular and papillary cystic spaces, and all surfaces were similar to the cuboidal and columnar epithelium of adenomas and of ductal epithelial hyperplasia.

LEVITT M.H.[8] mentioned that early neoplastic changes in all pancreatic cellular elements were followed by a progressive proliferation of intra- and interlobular duct cells, with the development of multicentric foci of cystic and papillary cystic adenomas, intraductal carcinomas, and invasive ductal neoplasms.

POUR P.[11,13] mentioned as follows. Lesions were found in intrapancreatic and extrapancreatic ducts. Equivalent alterations consisting of hyperplasia, metaplasia, atypia, and lesions characteristic of carcinoma in situ developed ubiquitously and simultaneously in pancreatic ducts of different sizes and in ductules, but not in acinar cells. Among the most significant findings were intrainsular ductular formations, their proliferation, and sequential malignant alteration comparable to the involved preexisting ductules. Ductular cells, especially those of periinsular origin, are the most responsive to BOP.

TAKAHASHI M.[19] mentioned that among 75 induced adenocarcinomas, most were of ductular origin, wheres only a few seemed to arise from ductal epithelium.

In our experiment, following facts were observed. Very early alterations were enlargements of the islets and focal hyperplasia of the ductules and ducts. These lesions seemed to become multilayer hyperplasia. At late weeks, in every dose, multiplicative lesions appeared at the islets and ductules. In our experiment, pseudoductular transformation of exocrine acini was first discovered. It is seen in Guinea pig pancreatic cancer induced by N-methyl-N-nitrosourea[16]. These lesions extended to whole pancreas. In some instances, the cells of lining epithelium of some these lesions became atypical and malignant[9]. These lesions were surrounded by destructed parenchymal tissue, lymphocytic reaction and desmoplastic reaction. Such phenomenon was thought to be caused by pressure of these lesions, lytic enzymes excreted from malignant cells and unknown etiology[5]. This is thought to be most early figure of cancerous tissue. Then, cancer cells spreading to destructed parenchymal tissue seemed to form glandular structures of adenocarcinoma. In succession, early small adenocarcinoma composed of glands and immature desmoplastic reaction appeared. Finally, adenocarcinomas of various differentiation with mature desmoplastic reaction appeared. The spreading of intraductal carcinoma of some interlobular ductules and large ducts was seen at even early weeks. But, they were not ac-

companied with neither destructed parenchymal tissue nor desmoplastic reaction. Accordingly, almost all adenocarcinomas of hamster pancreatic cancer induced by DHPN was thought to originate from malignant multiplication of intralobular ductules. A few of them were thought to occur from the spread of intraductal carcinoma of ducts.

In human pacreatic cancer, the correlation between atypical hyperplasia of pancreatic duct and adenocarcinoma was given attention[17,20]. From which components adenocarcinoma does occur is not known[1,3,4]. The facts obtained by analysis of the histogenesis of hamster pancreatic cancer must contribute to analysis of the histogenesis of human pancreatic cancer.

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和文抄録

N-nitroso-bis(2-hydroxypropyl)amine 誘発ハムスター膵癌の組織発生

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シリアンゴールデンハムスターの1群には 125 mg/kg 体重，2群には 250 mg/kg 体重，3群には 500 mg/kg 体重の N-nitroso-bis(2-hydroxypropyl)amine が毎週皮下注射され，5週後，10週後，15週後，25週後，35週後或いは，頻死の時屠殺され，病理学的に又，組織病理学的に観察された．

早期には，ラ氏島肥大や導管の部分的 unilayer hyperplasia が現われ，multilayer hyperplasia へと進行するとともに，その病変は膵全体に現われるようになった．続いて，multiplicative な病変が，ラ氏島や小葉内導管に現われた．その病変は腺房細胞にも見られることか，この実験で初めて明らかにされた．殆んど全ての腺癌は，悪性化した細胞よりなる multiplication より発生するものと考えられた．極く僅かの腺癌は，小葉間導管，主膵管の intraductal carcinoma の浸潤により発生するものと考えられた．